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Lymphocyte subsets and disease activity in treated juvenile-onset systemic lupus erythematosus: An exploratory single-center cross-sectional analysis

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Abstract

Juvenile-onset systemic lupus erythematosus (jSLE) is a severe autoimmune disease with high disease activity and multi-organ involvement. The relationship between lymphocyte subsets and disease activity in treated pediatric patients remains unclear. This study aimed to characterize lymphocyte subset distributions in treated jSLE and to explore their cross-sectional association with concurrent disease activity. This retrospective, single-center, exploratory study included patients who met the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria. Lymphocyte subsets were measured once at the last routine follow-up after treatment initiation; disease activity at the same visit was assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Associations were evaluated using Spearman correlation analysis with false discovery rate (FDR) adjustment. All eligible patients with complete data were included without an a priori sample size calculation. Fourteen patients (78.6% female) were analyzed. Median SLEDAI-2K decreased from 8.0 at diagnosis to 2.0 at the last visit ($p < 0.001$). Anti-double-stranded DNA (anti-dsDNA) decreased from 51.5 to 15.0 IU/mL ($p < 0.001$), erythrocyte sedimentation rate (ESR) from 42.0 to 14.5 mm/h ($p < 0.001$), and C-reactive protein (CRP) from 5.0 to 3.0 mg/L ($p = 0.013$). Absolute lymphocyte counts increased numerically from 1.7 to $2.2 \times 10^9/L$, but the difference was not statistically significant ($p = 0.060$; FDR $q = 0.083$). No robust FDR-adjusted correlations were found between the evaluated major lymphocyte subsets and concurrent SLEDAI-2K. CD20+ B-cell percentages were lower in patients exposed to intravenous immunoglobulin (IVIG), but the difference was not significant after FDR adjustment. In this small exploratory cohort of treated jSLE, single-time-point lymphocyte subset distributions were not robustly associated with concurrent disease activity. Immunophenotyping should be interpreted with clinical and laboratory parameters rather than as a standalone disease activity biomarker.

Keywords: Systemic lupus erythematosus; child; lymphocyte subsets; severity of illness index; immunophenotyping; flow cytometry

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by loss of immune tolerance, production of autoantibodies, and multi-organ involvement [1]. The disease results from a complex interplay of genetic susceptibility, environmental triggers, and dysregulation of both innate and adaptive immune responses [1-4]. Current classification of SLE

is based on the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria, which are widely used in both clinical practice and research settings [5].

Juvenile-onset systemic lupus erythematosus (jSLE), defined as disease onset before 18 years of age, represents a distinct clinical entity with a more severe phenotype compared to adult-onset

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disease. Patients with jSLE often exhibit higher disease activity, increased frequency of organ involvement, and a more aggressive disease course [6,7]. These differences highlight the need for a better understanding of disease mechanisms and biomarkers specifically in pediatric populations [6].

The immunopathogenesis of SLE involves profound alterations in lymphocyte subsets, including dysregulation of CD4+ T cells and hyperactivation of B cells, leading to autoantibody production and immune complex formation [3,4,8]. Studies in predominantly adult SLE cohorts have reported associations between peripheral T- and B-cell subsets and disease activity [9], while B-cell-focused literature emphasizes substantial heterogeneity across disease phenotypes and treatment settings [10]. However, data in pediatric populations remain limited.

Importantly, most existing studies have focused on adult patients or have evaluated immunological parameters without considering the potential impact of ongoing immunosuppressive treatment. Because treated patients often receive multiple immunomodulatory therapies included in current SLE management recommendations [11,12], treatment exposure may obscure the relationship between immune cell distributions and disease activity. Therefore, the clinical utility of lymphocyte subset analysis as a biomarker of disease activity in treated jSLE remains unclear. This gap is particularly relevant in pediatric populations, where disease heterogeneity and treatment exposure may further complicate biomarker interpretation.

In addition, monogenic forms of SLE, such as those associated with protein kinase C delta (PRKCD) mutations, have been increasingly recognized and may present with distinct immunological and clinical features compared to polygenic SLE [13,14]. These cases provide unique insights into disease mechanisms and further highlight the heterogeneity of lupus.

Given these considerations, there is a need to better characterize immunological profiles in jSLE and to evaluate their relationship with disease activity, particularly in treated pediatric cohorts in routine clinical practice.

Therefore, this study aimed to characterize the immunological profile of treated patients with jSLE and to explore whether lymphocyte subsets are associated with concurrent disease activity in a single-center cross-sectional analysis.

Material and Methods

Study Design and Population

This study was designed as a retrospective, single-center exploratory study with longitudinal clinical evaluation and cross-sectional immunological assessment in the Pediatric Rheumatology Department of Samsun City Hospital. Patients diagnosed with jSLE, defined as disease onset before 18 years of age, were included.

The diagnosis of SLE was established according to the 2019 EULAR/ACR classification criteria, which are widely used in both clinical practice and research settings [5]. Patients with incomplete clinical, laboratory, or immunological data were excluded from the analysis.

A total of 15 patients were initially identified. One patient with monogenic SLE due to a PRKCD mutation was analyzed separately and excluded from statistical analyses to avoid confounding effects, as monogenic lupus represents a biologically distinct entity [13,14].

Data Collection

Demographic, clinical, laboratory, and immunological data were retrospectively collected from electronic medical records. Recorded variables included age, sex, disease duration, follow-up time, and major organ involvement, such as renal manifestations. Renal involvement was defined as biopsy-proven lupus nephritis. All patients with renal involvement had class IV lupus nephritis.

Laboratory parameters included lymphocyte count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Serological markers included complement component 3 (C3) and complement component 4 (C4) levels and anti-double-stranded DNA (anti-dsDNA) antibody levels.

Disease Activity Assessment

Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), a validated and widely used tool for measuring disease activity in patients with SLE [15].

Baseline was defined as the time of diagnosis (initial presentation). Longitudinal changes in disease activity and laboratory parameters were evaluated by comparing baseline and last visit measurements. The cross-sectional immunological assessment was performed at the last routine follow-up visit after initiation of standard-of-care treatment. The SLEDAI-2K score recorded at the same visit as lymphocyte subset testing was used for cross-sectional association analyses. Although all samples were obtained at a routine follow-up visit, the clinical disease state at that visit was not standardized as flare or remission; this heterogeneity was considered when interpreting the results.

Immunological Assessment

Lymphocyte subset analysis was performed using flow cytometry on peripheral blood samples according to standardized laboratory protocols. All samples were analyzed using the same instrument and consistent technical procedures.

Immunological parameters included CD4+ and CD8+ T cells, CD19+ and CD20+ B cells, natural killer (NK) cells, and naïve, memory, and CD21^{low} B-cell subpopulations.

Lymphocyte subset analysis was performed at a single time point during routine follow-up. Associations between immunological parameters and disease activity were therefore evaluated cross-sectionally in relation to concurrent SLEDAI-2K scores, rather than as longitudinal immunological trajectories.

Treatment Variables

All patients were receiving standard-of-care treatment at the time of immunological evaluation, including hydroxychloroquine. Exposure to immunosuppressive therapies, including mycophenolate mofetil, azathioprine, methotrexate, intravenous immunoglobulin (IVIG), and cyclophosphamide, was reviewed. For statistical comparisons, treatment variables were defined as binary indicators reflecting exposure (ever vs never), because cumulative dose, exact timing in relation to immunophenotyping, and treatment duration were not uniformly available for all agents.

Outcomes

The primary outcome of the study was the evaluation of the association between lymphocyte subsets and disease activity at the time of immunological assessment.

Secondary outcomes included longitudinal changes in disease activity and serological markers.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as median and interquartile range (IQR), while categorical variables were presented as counts and percentages.

Due to the non-normal distribution of the data, non-parametric methods were used. Paired comparisons between baseline and last visit measurements were performed using the Wilcoxon signed-rank test [16].

Correlations between selected major lymphocyte subsets—CD4+ T cells, CD8+ T cells, and CD19+ B cells—and concurrent SLEDAI-2K scores at the time of lymphocyte subset analysis were evaluated using Spearman correlation analysis.

Subgroup comparisons were conducted using the Mann–Whitney U test.

To account for multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) procedure was applied separately to longitudinal comparisons, correlation analyses, and exploratory IVIG subgroup comparisons. FDR-adjusted p-values (q-values) < 0.05 were considered statistically significant.

Sample Size and Power Considerations

No formal a priori sample-size calculation was performed because this retrospective study included all eligible jSLE patients with complete clinical, laboratory, and immunological data during the study period. An approximate sensitivity

analysis based on a conventional two-sided correlation test indicated that, with 14 patients and an alpha level of 0.05, approximately 80% power would be available only for detecting a large correlation of about $|r| = 0.66$ or greater. Because the primary association analyses used Spearman correlation and the sample size was small, this estimate should be interpreted as approximate. Therefore, the absence of statistical significance does not exclude small-to-moderate associations, and the correlation analyses were considered exploratory and hypothesis-generating rather than confirmatory. Treatment subgroup comparisons were also considered exploratory because of the small group sizes.

Ethical Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki [17]. Ethical approval was obtained from the Samsun University Non-Interventional Clinical Research Ethics Committee (Approval No: GOKAEK 2026/7/4; Date: 22 April 2026). Written informed consent was obtained from all patients and/or their legal guardians prior to inclusion in the study.

Results

Demographic and Clinical Characteristics

A total of 15 patients were initially identified. After excluding one patient with monogenic SLE, analyses were performed on 14 patients with juvenile-onset SLE (jSLE). Among these patients, 11 (78.6%) were female and 3 (21.4%) were male. The median age at diagnosis was 15.2 years (IQR: 11.9–16.1), and the median disease duration was 2.2 years (IQR: 1.5–3.0) (Table 1).

Table 1. Demographic and clinical characteristics of the study population (n = 14)

Variable	Value
Female, n (%)	11 (78.6%)
Male, n (%)	3 (21.4%)
Age at diagnosis, years	15.2 (11.9–16.1)
Disease duration, years	2.2 (1.5–3.0)

Renal involvement was present in 2 patients, both of whom had biopsy-proven class IV lupus nephritis.

Longitudinal Changes in Disease Activity and Laboratory Parameters

Median SLEDAI-2K scores were 8.0 (IQR: 6.3–11.5) at baseline and 2.0 (IQR: 2.0–3.8) at the last visit ($p < 0.001$) (Table 2).

Anti-dsDNA levels were 51.5 IU/mL (IQR: 29.0–110.3) at baseline and 15.0 IU/mL (IQR: 10.1–21.4) at the last visit ($p < 0.001$) (Table 2).

CRP values were 5.0 (3.0–7.5) mg/L at baseline and 3.0 (1.0–3.2) mg/L at the last visit ($p = 0.013$), and ESR values were 42.0 (20.3–65.0) mm/h and 14.5 (8.0–20.0) mm/h, respectively ($p < 0.001$) (Table 2).

Absolute lymphocyte counts were $1.7 (1.2–2.2) \times 10^9/L$ at baseline

and $2.2 (1.7–2.5) \times 10^9/L$ at the last visit ($p = 0.060$) (Table 2).

C3 levels were 1.15 (0.73–1.30) g/L at baseline and 1.20 (1.00–1.28) g/L at the last visit ($p = 0.528$), and C4 levels were 0.15 (0.08–0.20) g/L and 0.20 (0.13–0.21) g/L, respectively ($p = 0.638$) (Table 2).

Table 2. Longitudinal changes in disease activity and laboratory parameters

Variable	Baseline Median (IQR)	Last Visit Median (IQR)	p-value	FDR-q
SLEDAI-2K	8.0 (6.3–11.5)	2.0 (2.0–3.8)	< 0.001	0.002
CRP (mg/L)	5.0 (3.0–7.5)	3.0 (1.0–3.2)	0.013	0.023
ESR (mm/h)	42.0 (20.3–65.0)	14.5 (8.0–20.0)	< 0.001	0.002
ALC ($\times 10^9/L$)	1.7 (1.2–2.2)	2.2 (1.7–2.5)	0.060	0.083
Anti-dsDNA (IU/mL)	51.5 (29.0–110.3)	15.0 (10.1–21.4)	< 0.001	<0.001
C3 (g/L)	1.15 (0.73–1.30)	1.20 (1.00–1.28)	0.528	0.617
C4 (g/L)	0.15 (0.08–0.20)	0.20 (0.13–0.21)	0.638	0.638

Abbreviations: SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALC, absolute lymphocyte count; anti-dsDNA, anti-double-stranded DNA; C3, complement component 3; C4, complement component 4; IQR, interquartile range; FDR, false discovery rate. Values are presented as median (IQR). Paired comparisons were performed using the Wilcoxon signed-rank test with FDR adjustment.

Immunological Profile

The median CD4+ T cell level was 36.0% (IQR: 30.8–40.8), CD8+ T cell level was 30.0% (IQR: 27.5–45.0), and CD19+ B cell level was 13.0% (IQR: 8.5–15.8). Median CD20+ B cell levels were 11.0% (IQR: 8.0–14.0), and NK cell levels were 8.0% (IQR: 4.8–11.0). Naïve B cells were 84.0% (IQR: 73.0–86.0), memory B cells were 7.0% (IQR: 6.0–16.0), and CD21^{low} B cells were 3.0% (IQR: 0.0–5.0) (Table 3).

Table 3. Immunological profile of patients with jSLE

Parameter	Median (IQR)
CD4+ T cells (%)	36.0 (30.8–40.8)
CD8+ T cells (%)	30.0 (27.5–45.0)
CD19+ B cells (%)	13.0 (8.5–15.8)
CD20+ B cells (%)	11.0 (8.0–14.0)
NK cells (%)	8.0 (4.8–11.0)
Naïve B cells (%)	84.0 (73.0–86.0)
Memory B cells (%)	7.0 (6.0–16.0)
CD21 ^{low} B cells (%)	3.0 (0.0–5.0)

Abbreviations: jSLE, juvenile-onset systemic lupus erythematosus; NK, natural killer; IQR, interquartile range.

Association Between Lymphocyte Subsets and Disease Activity

No statistically significant correlations were identified between the evaluated CD4+ T-cell, CD8+ T-cell, and CD19+ B-cell percentages and concurrent SLEDAI-2K scores. Correlation coefficients were as follows: CD4+ T cells ($r = -0.37$, $p = 0.24$; FDR $q = 0.36$), CD8+ T cells ($r = 0.17$, $p = 0.59$; FDR $q = 0.59$), and CD19+ B cells ($r = -0.47$, $p = 0.093$; FDR $q = 0.279$) (Table 4).

Table 4. Correlations between lymphocyte subsets and SLEDAI-2K scores

Parameter	Correlation coefficient (r)	p-value	FDR-q
CD4+ T cells vs SLEDAI-2K	-0.37	0.24	0.36
CD8+ T cells vs SLEDAI-2K	0.17	0.59	0.59
CD19+ B cells vs SLEDAI-2K	-0.47	0.093	0.279

Abbreviations: SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; FDR, false discovery rate.

Treatment Characteristics

All patients were receiving hydroxychloroquine therapy. Seven patients had been exposed to mycophenolate mofetil (50.0%), five to IVIG (35.7%), one to cyclophosphamide (7.1%), three to azathioprine (21.4%), and two to methotrexate (14.3%) (Table 5).

Table 5. Treatment exposure in the study population

Treatment	n (%)
Mycophenolate mofetil (MMF)	7 (50.0%)
Intravenous immunoglobulin (IVIG)	5 (35.7%)
Cyclophosphamide	1 (7.1%)
Azathioprine	3 (21.4%)
Methotrexate	2 (14.3%)

Abbreviations: MMF, mycophenolate mofetil; IVIG, intravenous immunoglobulin. Treatment variables represent previous exposure at any time before or at immunological assessment. All patients were receiving hydroxychloroquine at the time of immunological evaluation.

Monogenic SLE Case

The patient with monogenic SLE due to a PRKCD mutation was evaluated separately. The SLEDAI-2K score was 12 at baseline and 4 at the last visit. CD4+ T-cell, CD8+ T-cell, and CD19+ B-cell percentages were 23%, 36%, and 6%, respectively. Naïve B cells accounted for 99%, and CD21^{low} B cells accounted for 19%. Anti-dsDNA levels decreased from 97 IU/mL at baseline to 2.4 IU/mL at the last visit. C3 levels increased from 0.5 to 1.2 g/L, while C4 levels remained at 0.2 g/L at both time points. The patient had class IV lupus nephritis and had previously received IVIG and six cycles of cyclophosphamide before initiation of mycophenolate mofetil, in addition to corticosteroid therapy (Table 6).

Table 6. Clinical and immunological features of the monogenic SLE case

Parameter	Value
Gene	PRKCD
CD4+ T cells (%)	23
CD8+ T cells (%)	36
CD19+ B cells (%)	6
Naïve B cells (%)	99
CD21 ^{low} (%)	19
SLEDAI-2K score	Baseline: 12; Last visit: 4
Anti-dsDNA (IU/mL)	Baseline: 97; Last visit: 2.4
C3 (g/L)	Baseline: 0.5; Last visit: 1.2
C4 (g/L)	Baseline: 0.2; Last visit: 0.2
Renal involvement	Class IV lupus nephritis

Abbreviations: PRKCD, protein kinase C delta; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; anti-dsDNA, anti-double-stranded DNA; C3, complement component 3; C4, complement component 4.

Subgroup Analysis According to IVIG Exposure

CD4+ T cell levels were 28% (20.75–36) in the IVIG group and 38.5% (34.75–43.5) in the non-IVIG group (p = 0.234).

CD8+ T cell levels were 37% (26.25–46.75) and 30% (27.5–36.75), respectively (p = 1.000).

CD19+ B cell levels were 8% (6–14) in the IVIG group and 14% (12–16) in the non-IVIG group (p = 0.229).

CD20+ B cell levels were 8% (6–10) in the IVIG group and 13% (11.75–16.25) in the non-IVIG group (unadjusted p = 0.023; FDR q = 0.092) (Table 7).

Table 7. Exploratory comparison of lymphocyte subsets according to IVIG exposure

Parameter	IVIG (n = 5), Median (IQR)	No IVIG (n = 9), Median (IQR)	p-value	FDR-q
CD4+ T cells (%)	28 (20.75–36)	38.5 (34.75–43.5)	0.234	0.312
CD8+ T cells (%)	37 (26.25–46.75)	30 (27.5–36.75)	1.000	1.000
CD19+ B cells (%)	8 (6–14)	14 (12–16)	0.229	0.312
CD20+ B cells (%)	8 (6–10)	13 (11.75–16.25)	0.023	0.092

Abbreviations: IVIG, intravenous immunoglobulin; IQR, interquartile range; FDR, false discovery rate. Subgroup comparisons were exploratory. Group sizes are shown in the column headings; analyses were performed using available data for each lymphocyte subset. The lower CD20+ B-cell percentage in IVIG-exposed patients was nominally significant before multiple-comparison adjustment but did not remain statistically significant after FDR adjustment.

Discussion

In this study, we evaluated the immunological profile of treated jSLE patients and examined the exploratory cross-sectional relationship between lymphocyte subsets and concurrent disease activity in a single-center pediatric cohort. The main findings were as follows: (i) significant longitudinal improvement in disease activity and serological markers was observed, (ii) lymphocyte subset distributions demonstrated substantial inter-individual variability, and (iii) no robust FDR-adjusted cross-sectional associations were identified between lymphocyte subsets and concurrent SLEDAI-2K scores.

The observed reductions in SLEDAI-2K scores and anti-dsDNA levels during follow-up indicate improved disease control in this cohort. Anti-dsDNA antibodies and complement levels are well-established serological biomarkers of SLE activity [18,19]. Similarly, reductions in CRP and ESR further support the overall decrease in inflammatory burden in our cohort.

In contrast to the clear improvement in clinical and serological parameters, no robust FDR-adjusted correlations were identified between the evaluated lymphocyte subsets and

concurrent disease activity. Because the study did not include an age-matched healthy control group and lymphocyte subsets were assessed predominantly as relative percentages, the present data cannot determine whether individual cell populations were increased or decreased compared with healthy children. Concurrent disease activity was also generally low and narrowly distributed, with a median SLEDAI-2K score of 2.0 (IQR: 2.0–3.8), which may have reduced the ability to detect cross-sectional associations. The inverse correlations observed for CD4+ T cells and CD19+ B cells did not reach statistical significance after FDR adjustment. Notably, the correlation between CD19+ B-cell percentage and SLEDAI-2K was moderate in magnitude ($r = -0.47$; FDR $q = 0.279$), but the estimate was imprecise and did not remain statistically significant after FDR adjustment. This finding should therefore be interpreted cautiously and requires confirmation in larger longitudinal studies. Previous studies have reported associations between selected lymphocyte subsets and disease activity, whereas B-cell-focused studies and reviews have emphasized substantial heterogeneity according to disease phenotype, activity, treatment exposure, and analytical approach [8-10,20]. These findings support cautious interpretation of single-time-point flow-cytometric lymphocyte subset measurements as disease activity biomarkers in treated jSLE.

Several factors may explain the lack of meaningful correlations observed in our study. First, lymphocyte subsets were assessed at a single routine follow-up visit and analyzed in relation to concurrent disease activity. This design does not capture dynamic immunological changes occurring at diagnosis, during flare, during remission, or after treatment modifications. Second, all patients were receiving active treatment at the time of immunological assessment. Hydroxychloroquine was used in all patients, and several patients had been exposed to mycophenolate mofetil, azathioprine, methotrexate, IVIG, cyclophosphamide, and corticosteroids. These therapies have distinct immunomodulatory effects and may influence lymphocyte abundance and function. Conventional SLE therapies affect immune-cell activation and proliferation [11,12,20], whereas IVIG has multiple immunomodulatory effects involving Fc-receptor signaling, autoantibodies, and B-cell responses [21]. Therefore, observed lymphocyte profiles may reflect treatment exposure as much as intrinsic disease activity. Because cumulative dose, timing, and treatment duration were not uniformly available, treatment effects could not be fully adjusted for in this cohort.

SLE is a highly dynamic disease characterized by fluctuating immune activity, and cross-sectional measurements may not adequately capture temporal changes in immune cell populations [3,4,20]. Accordingly, longitudinal within-patient immunophenotyping may be more informative than a single cross-sectional measurement; this represents a rationale for future study rather than a conclusion established by the present data. Therefore, the lack of association observed in our study should not be interpreted as absence of biological relevance, but

rather as a limitation of static immune profiling in a dynamic and treatment-modified disease.

Third, the relatively small sample size and clinical variability of the cohort limited the statistical power to detect subtle associations. With 14 analyzed patients, the study had limited ability to detect small-to-moderate correlations; therefore, negative findings should be interpreted as inconclusive for such associations rather than definitive evidence of no biological relationship. Pediatric SLE is known for its heterogeneity, both clinically and immunologically, which may further contribute to variability in immune cell profiles [6,7].

CD19+ and CD20+ B-cell percentages were both lower in IVIG-exposed patients, indicating a directionally concordant pattern. However, only the CD20+ comparison was nominally significant, and this association did not remain statistically significant after FDR adjustment (unadjusted $p = 0.023$; FDR $q = 0.092$). Although IVIG has immunomodulatory effects on B-cell activation and antibody responses [21], it is not a B-cell-depleting treatment, and the present cross-sectional data do not support a direct causal effect of IVIG on circulating B-cell percentages. Alternative explanations include confounding by indication, greater underlying disease or treatment burden among patients who received IVIG, concomitant exposure to corticosteroids or other immunosuppressive agents, variation in the interval between IVIG administration and immunophenotyping, and sampling variability due to the very small subgroup sizes. Accordingly, this finding should be regarded as a preliminary treatment-associated signal that requires prospective confirmation rather than evidence of an IVIG-specific effect.

The inclusion of a patient with monogenic SLE due to a PRKCD mutation represents a unique aspect of our study. This case was not included in correlation analyses. This patient demonstrated a distinct immunological profile, consistent with previous reports indicating that monogenic lupus differs from polygenic SLE in both pathogenesis and immune phenotype [13,14]. This observation further supports the concept of immunological heterogeneity in SLE and highlights the importance of individualized approaches. Additionally, it suggests that distinct immunopathogenic mechanisms may underlie different forms of SLE, limiting the generalizability of immunological biomarkers across patient subgroups. Although classical features of immunodeficiency were not prominent, the observed lymphocyte profile, including the CD4+ T-cell and B-cell findings, may reflect underlying immune dysregulation consistent with inborn errors of immunity associated with monogenic forms of SLE. This may also be influenced by prior exposure to intensive immunosuppressive therapies.

Taken together, our findings suggest that, in a small treated pediatric cohort, conventional lymphocyte subset analysis may have limited standalone value for cross-sectional disease activity assessment. However, this should be interpreted as a hypothesis-

generating observation rather than a definitive conclusion. Flow cytometry remains valuable for characterizing immune system alterations, but its role in routine disease activity monitoring in treated jSLE requires longitudinal validation. These findings also highlight the need for dynamic immune-monitoring approaches that account for treatment exposure, disease stage, and within-patient temporal changes.

This study has several limitations. The retrospective, single-center design and small sample size limit generalizability and reduce the statistical power to detect subtle but clinically meaningful associations. No formal a priori sample-size calculation was performed, and the approximate sensitivity analysis indicated limited ability to detect small-to-moderate correlations. Single-time-point cross-sectional immunophenotyping is a major limitation because SLE is a dynamic disease and immune profiles may fluctuate over time. The timing of immunophenotyping was not standardized to diagnosis, flare, remission, or treatment change; instead, lymphocyte subsets were assessed at routine follow-up visits under active treatment. Furthermore, treatment exposure was heterogeneous, and cumulative dose, duration, and exact timing relative to flow-cytometric testing were not uniformly available, limiting the ability to adjust for treatment effects. Accordingly, the cross-sectional design precludes assessment of intra-individual immunological fluctuations over time, which may be more informative than isolated absolute measurements.

Conclusion

In conclusion, while clinical and serological parameters improved significantly in treated jSLE patients, lymphocyte subset distributions showed marked variability and did not demonstrate robust FDR-adjusted cross-sectional associations with concurrent disease activity in this small exploratory cohort. These findings should be considered hypothesis-generating and suggest that single-time-point immunophenotyping should not be used as a standalone disease activity measure. Instead, lymphocyte subset data should be interpreted together with clinical and laboratory parameters within individualized patient assessment. Future longitudinal, adequately powered studies are needed to define whether dynamic immune profiling can contribute to disease monitoring in pediatric SLE.

Ethical Approval

Ethical approval was obtained from the Samsun University Non-Interventional Clinical Research Ethics Committee (Approval No: GOKAEK 2026/7/4; Date: 22 April 2026).

Patient Consent

Written informed consent was obtained from all patients and/or their legal guardians prior to inclusion in the study.

Conflict of Interest

The authors confirm the absence of any conflicts associated with this work.

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References

1. Rahman A, Isenberg DA. Systemic lupus erythematosus. *N Engl J Med*. 2008;358:929-39.
2. Kaul A, Gordon C, Crow MK, et al. Systemic lupus erythematosus. *Nat Rev Dis Primers*. 2016;2:16039.
3. Tsokos GC, Lo MS, Costa Reis P, Sullivan KE. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol*. 2016;12:716-30.
4. Tsokos GC. Autoimmunity and organ damage in systemic lupus erythematosus. *Nat Immunol*. 2020;21:605-14.
5. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis*. 2019;78:1151-9.
6. Levy DM, Kamphuis S. Systemic lupus erythematosus in children and adolescents. *Pediatr Clin North Am*. 2012;59:345-64.
7. Brunner HI, Gladman DD, Ibañez D, et al. Difference in disease features between childhood-onset and adult-onset systemic lupus erythematosus. *Arthritis Rheum*. 2008;58:556-62.
8. Jenks SA, Cashman KS, Zumaquero E, et al. Distinct effector B cells induced by unregulated Toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. *Immunity*. 2018;49:725-39.e6. Erratum in: *Immunity*. 2020;52:203.
9. Feng Y, Li Z, Xie C, Lu F. Correlation between peripheral blood lymphocyte subpopulations and primary systemic lupus erythematosus. *Open Life Sci*. 2022;17:839-45.
10. Parodis I, Stockfelt M, Sjöwall C. B cell therapy in systemic lupus erythematosus: from rationale to clinical practice. *Front Med (Lausanne)*. 2020;7:316.
11. Fanouriakis A, Kostopoulou M, Alunno A, et al. 2019 update of the EULAR recommendations for the management of systemic lupus erythematosus. *Ann Rheum Dis*. 2019;78:736-45.
12. Fanouriakis A, Tziolos N, Bertsias G, Boumpas DT. Update on the diagnosis and management of systemic lupus erythematosus. *Ann Rheum Dis*. 2021;80:14-25.
13. Omarjee O, Picard C, Frachette C, et al. Monogenic lupus: dissecting heterogeneity. *Autoimmun Rev*. 2019;18:102361.
14. Belot A, Kasher PR, Trotter EW, et al. Protein kinase C δ deficiency causes Mendelian systemic lupus erythematosus with B cell-defective apoptosis and hyperproliferation. *Arthritis Rheum*. 2013;65:2161-71.
15. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol*. 2002;29:288-91.
16. Conover WJ. *Practical Nonparametric Statistics*. 3rd ed. New York: Wiley; 1999.
17. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human participants. *JAMA*. 2025;333:71-4.
18. Pisetsky DS. Anti-DNA antibodies—quintessential biomarkers of SLE. *Nat Rev Rheumatol*. 2016;12:102-10.
19. Leffler J, Bengtsson AA, Blom AM. The complement system in systemic lupus erythematosus: an update. *Ann Rheum Dis*. 2014;73:1601-6.
20. Dörner T, Furie R. Novel paradigms in systemic lupus erythematosus. *Lancet*. 2019;393:2344-58.
21. Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med*. 2001;345:747-55.